Supplementary Information

Title: Deep learning for the prediction of early on-treatment response in metastatic colorectal cancer from serial medical imaging

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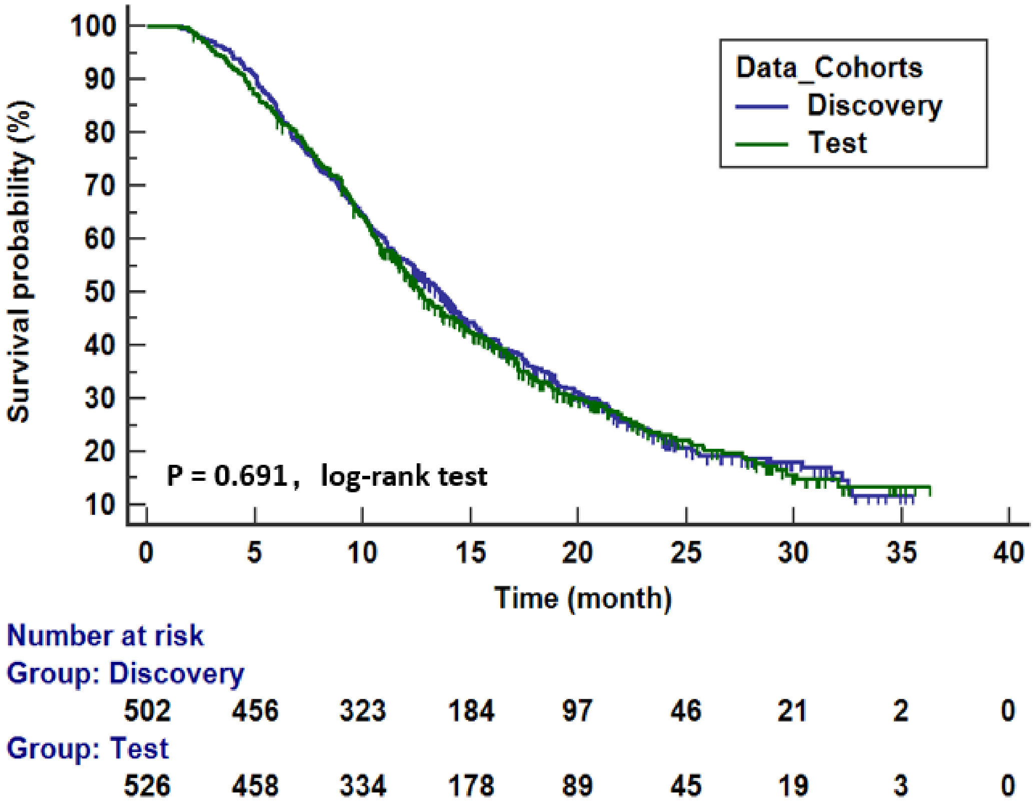
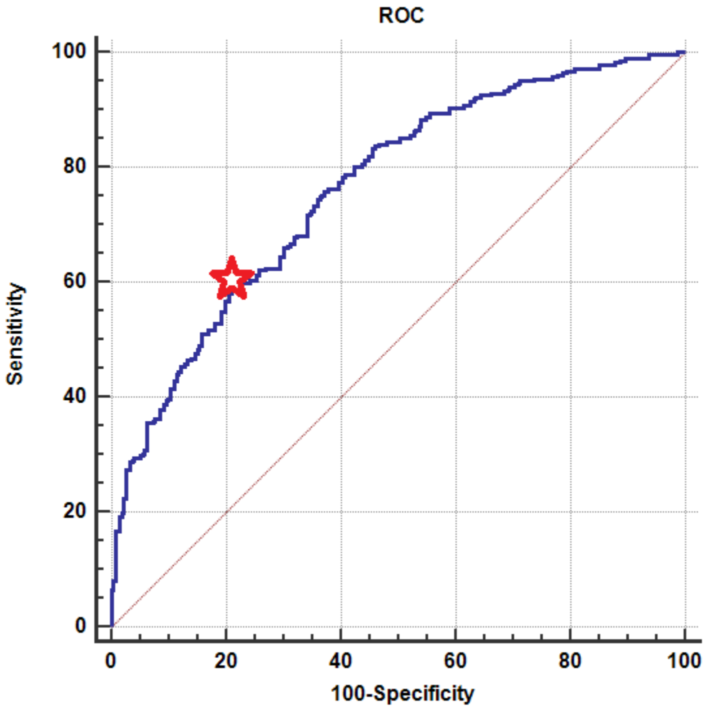


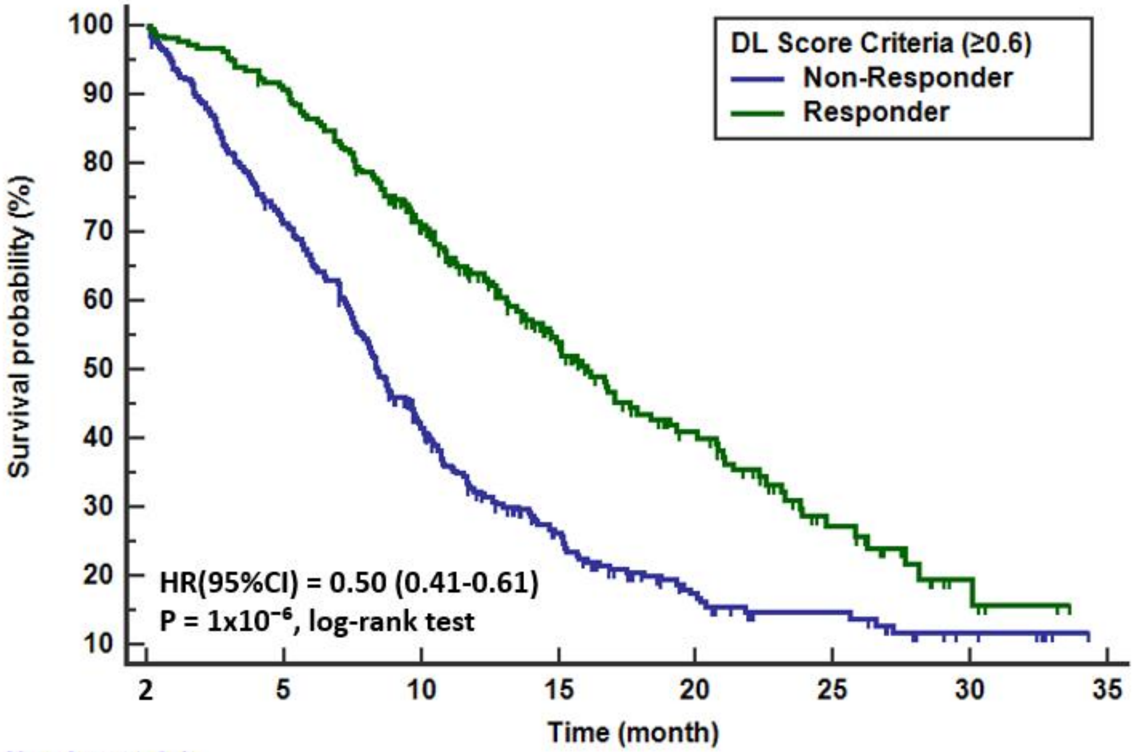
Number at risk

Group: Discovery								
502	456	323	184	97	46	21	2	0
Group: Test								
526	458	334	178	89	45	19	3	0

Supplementary Figure 1. Kaplan-Meier analysis for the discovery and test cohorts. The media OS for patients were 13.56 and 12.71 months in the discovery and test cohorts, respectively. There is no significant difference between the discovery and test cohorts (p = 0.691, log-rank test).



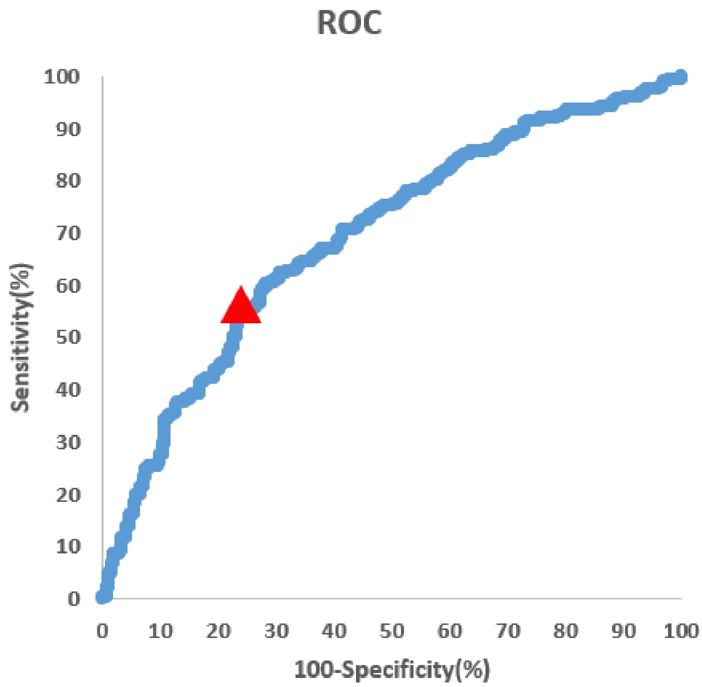
Supplementary Figure 2. The ROC of DL prediction score on the tuning set. The optimal point (indicated by the star) was found at ‘criterion: ≥0.6’, where the corresponding sensitivity and specificity were 60.6% and 78.6%, respectively.



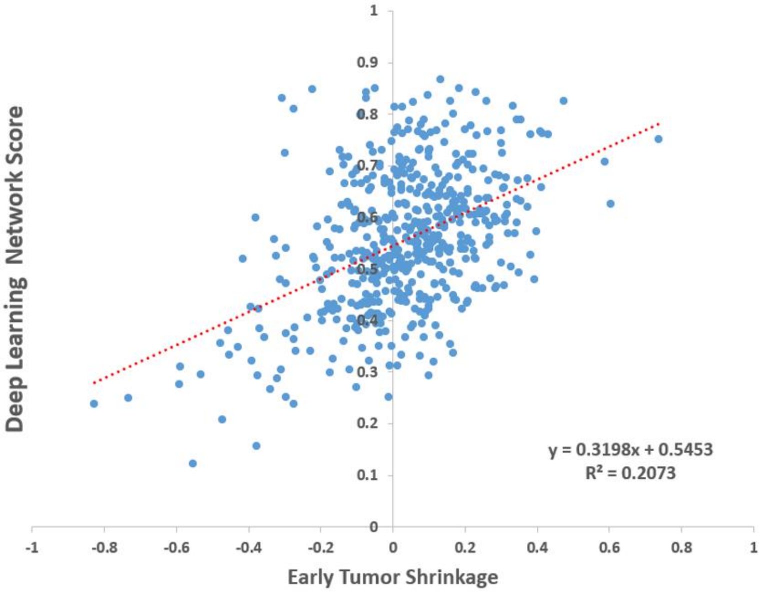
Number at risk

Group: Non-Responder	310	219	116	58	27	16	6	0
Group: Responder	216	195	140	81	45	18	5	0

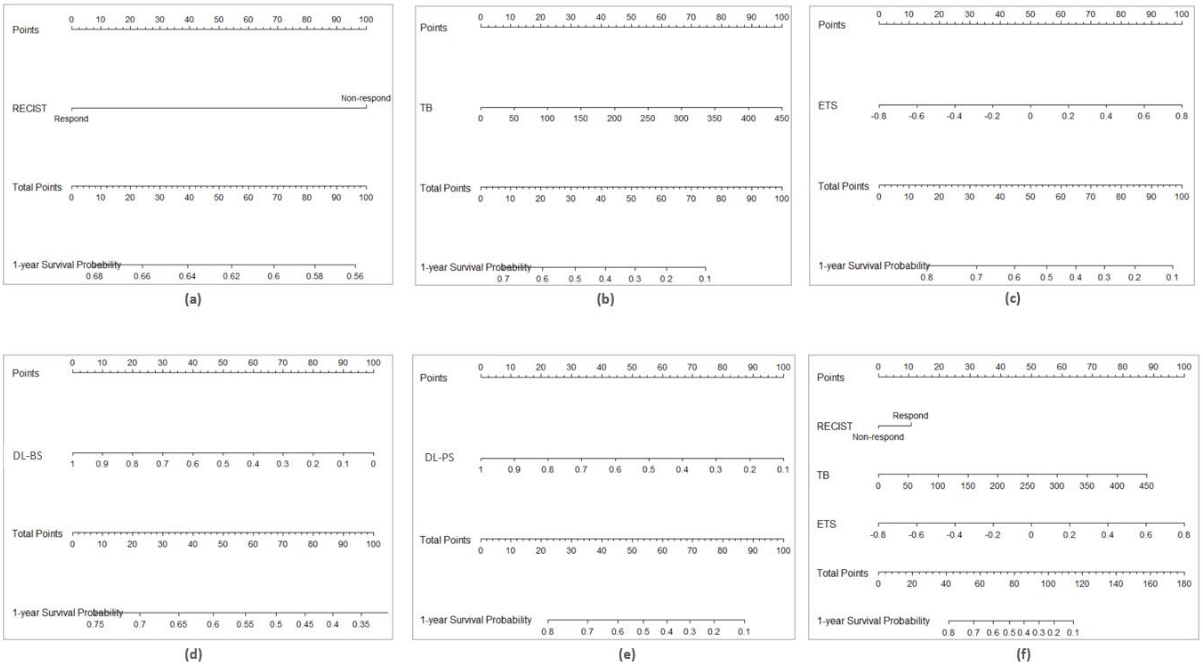
Supplementary Figure 3. The landmark analysis on the DL network starting on month-2.



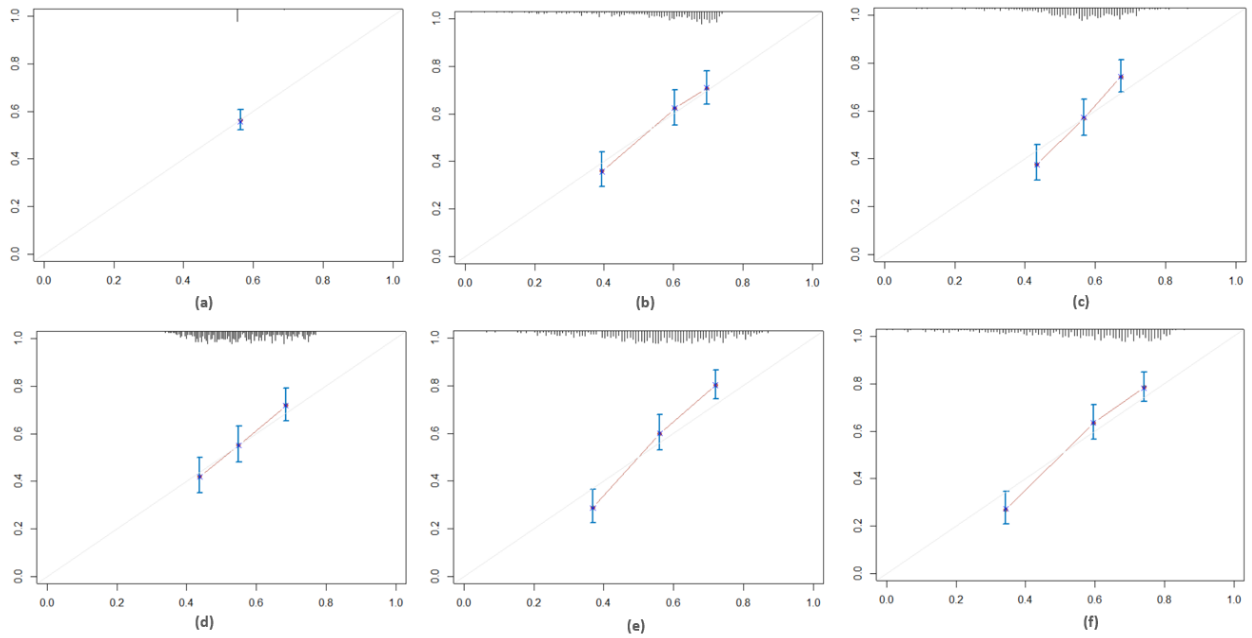
Supplementary Figure 4. The ROC of ETS on the tuning set. The optimal point (indicated by the star) was found at ‘criterion: ≥0.05’, where the corresponding sensitivity and specificity were 59.5% and 72.3, respectively.



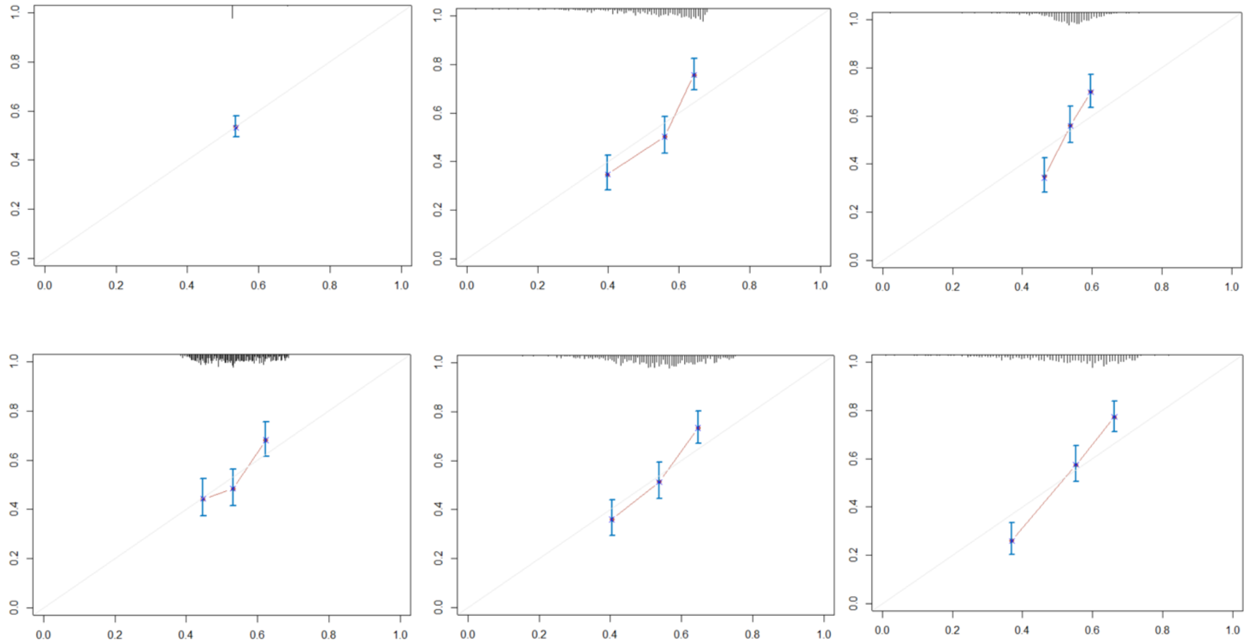
Supplementary Figure 5. Linear correlation between DL prediction score and ETS.



Supplementary Figure 6. (a-f) are the nomograms for the RECIST, TB, ETS, DL-BS, DL-PS and Size-Nomo models, respectively.



Supplementary Figure 7. (a-f) are the calibration curves in the discovery cohort (n = 502 patients) for the RECIST, TB, ETS, DL-BS, DL-PS and Size-Nomo models, respectively. The predicted survival probability for the three patient groups (The short, median, and long survival groups with patients of n = 502/3 in each group) were shown as error bars, i.e., mean value ± standard error.



Supplementary Figure 8. (a-f) are the calibration curves in the discovery cohort (n = 526 patients) for the RECIST, TB, ETS, DL-BS, DL-PS and Size-Nomo models, respectively. The predicted survival probability for the three patient groups (The short, median, and long survival groups with patients of n = 526/3 in each group) were shown as error bars, i.e., mean value ± standard error.

Supplementary Table

Supplementary Table 1. Summary of comparison between DL and ETS criteria.

Therapy & Line	Trail & Assessment Criterion	Treatment Arms	Patient Number	Criterion Res vs. Non-res	Respond Rate / Not Respond Rate		Median OS (months)			HR (95%CI)	p-value*
					Res	Non-res	Res	Non-res	Δ		
Chemo. ± anti-VEGF in 2nd line	VELOUR with optimal DL criterion	FOLFIRI + Afl.	259	Score ≥ 0.6 vs. <0.6	46%	55%	18.1	10.8	7.3	0.56 (0.41-0.76)	<0.001
		FOLFIRI	267	Score ≥ 0.6 vs. <0.6	37%	63%	18	10.2	7.8	0.45 (0.34-0.60)	<0.001
	VELOUR with optimal ETS criterion	FOLFIRI + Afl.	259	ETS ≥ 5% vs. <5%	59%	41%	16	10.6	5.4	0.57 (0.41-0.79)	<0.001
		FOLFIRI	267	ETS ≥ 5% vs. <5%	39%	62%	15.2	10.6	4.6	0.65 (0.49-0.86)	<0.001
	VELOUR with ETS criterion 20%	FOLFIRI + Afl.	259	ETS ≥ 20% vs. <20%	25%	75%	18.6	12	6.6	0.48 (0.35-0.67)	<0.001
		FOLFIRI	267	ETS ≥ 20% vs. <20%	11%	89%	17.2	11.8	5.4	0.61(0.42-0.89)	<0.001
	VELOUR with ETS criterion 30%	FOLFIRI + Afl.	259	ETS ≥ 30% vs. <30%	10%	90%	15.5	12.7	2.8	0.71 (0.44-1.15)	NS
		FOLFIRI	267	ETS ≥ 30% vs. <30%	4%	96%	27.6	11.9	15.7	0.52 (0.30-0.91)	NS
Chemo. ± anti-EGFR in 2nd line	BOND with optimal ETS criterion	Cet. + irinotecan	192	ETS ≥ 10% vs. <10%	37%	63%	12	7.4	4.6	0.33 (0.22-0.52)	<0.001
		Cet.	97	ETS ≥ 10% vs. <10%	28%	72%	na	na	na	na	na
	20050181 study with ETS criterion 30%	FOLFIRI+Pani.	181	ETS ≥ 30% vs. <30%	37%	63%	na	na	na	na	na
		FOLFIRI	180	ETS ≥ 30% vs. <30%	7%	93%	na	na	na	na	na
Chemo. ± anti-EGFR in 1st line	CRYSTAL with ETS criterion 20%	FOLFIRI + Cet.	299	ETS ≥ 20% vs. <20%	62%	38%	30	18.6	11.4	0.53 (0.40-0.69)	<0.001
		FOLFIRI	332	ETS ≥ 20% vs. <20%	49%	51%	24.1	18.6	5.5	0.71 (0.56-0.91)	0.006
	OPUS with ETS criterion 20%	FOLFOX-4 + Cet.	78	ETS ≥ 20% vs. <20%	69%	31%	26	15.7	10.3	0.43 (0.23-0.78)	0.006
		FOLFOX-4	90	ETS ≥ 20% vs. <20%	46%	54%	21.6	17.8	3.8	0.89 (0.53-1.47)	NS
	PRIME with ETS criterion 20%	FOLFOX-4 + Pani.	219	ETS ≥ 20% vs. <20%	72%	28%	32.5	12.6	19.9	0.47 (0.34-0.65)	<0.001
		FOLFOX-4	221	ETS ≥ 20% vs. <20%	57%	43%	26	15.2	10.8	0.50 (0.37-0.66)	<0.001
	PRIME with ETS criterion 30%	FOLFOX-4 + Pani.	219	ETS ≥ 30% vs. <30%	59%	41%	34.5	18.2	16.3	0.52 (0.38-0.70)	<0.001
		FOLFOX-4	221	ETS ≥ 30% vs. <30%	38%	62%	30.7	16	14.7	0.46 (0.34-0.63)	<0.001
Chemo. + anti-EGFR/VEGF in 1st line	FIRE-3 with ETS criterion 20%	FOLFIRI + Cet.	157	ETS ≥ 20% vs. <20%	68%	32%	38.3	20.5	17.8	0.52 (0.34-0.80)	0.002
		FOLFIRI + Bev.	173	ETS ≥ 20% vs. <20%	49%	51%	31.9	21.2	10.7	0.49 (0.35-0.71)	<0.001
	PEAK with ETS criterion 20%	mFOLFOX6 + Pani.	80	ETS ≥ 20% vs. <20%	75%	20%	43.4	21.2	22.2	0.38 (0.20-0.69)	<0.001
		mFOLFOX6 + Bev.	74	ETS ≥ 20% vs. <20%	62%	28%	32.5	21.8	10.7	0.42 (0.24-0.75)	<0.001
	PEAK with ETS criterion 30%	mFOLFOX6 + Pani.	80	ETS ≥ 30% vs. <30%	64%	36%	43.8	34.2	9.6	0.40 (0.22-0.72)	<0.001
		mFOLFOX6 + Bev.	74	ETS ≥ 30% vs. <30%	45%	55%	35.1	23.9	11.2	0.43 (0.24-0.78)	<0.001
	TRIBE with ETS criterion 20%	FOLFOXIRI + Bev.	225	ETS ≥ 20% vs. <20%	63%	30%	na	na	na	na	na
		FOLFIRI + Bev.	216	ETS ≥ 20% vs. <20%	52%	40%	na	na	na	na	na

Note: The p-values presented in the table were calculated via log-rank test and their numerical values were cited from the corresponding literatures.

Supplementary discussion on comparison between DL-based and size-based criteria. The comparison between DL and ETS criteria in the VELOUR trial as well as eight other well-known mCRC-related trials are summarized in Supplementary Table 1. The eight mCRC-related trials were found by conducting a literature search in the National Library of Medicine medical literature database via PubMed gateway with the key words 'early tumor shrinkage' or 'tumor size decrease' and 'metastatic colorectal cancer' (https://pubmed.ncbi.nlm.nih.gov/). The eight trails were BOND[1], 20050181 study [2], CRYSTAL [3], OPUS [3], PRIME [4], FIRE-3 [5], PEAK [6], and TRIBE [7], which including both phase II/III and first/second-line treatments. The data of the eight trails were cited from the corresponding published literatures. The comparison indicators included response rate (i.e., percentage of patients who responded to treatment), median OS and delta median OS (Δ), HR (95%CI), and p-value estimated by log-rank test.

Table 1 shows that our study was the first work to investigate early on-treatment prediction of outcome in mCRC patients receiving chemo. + anti-VEGF as a second-line treatment. In the VELOUR trial, the criteria "DL Score ≥ 0.6", "ETS ≥ 5%", and "ETS ≥ 20%" were all able to significantly stratify patients into responders and non-responders at the month-2 time point ($p<0.001$). Compared to the "ETS ≥ 5%", the "DL Score ≥ 0.6" showed superior performance in terms of a larger delta median OS, with 7.3 vs. 5.4 (Δ month) and 7.8 vs. 4.6 (Δ month) in the FA and F arms, respectively. Compared to the "ETS ≥ 20%", the "DL Score ≥ 0.6" showed superior performance in terms of higher response rate, with 46% vs. 25% and 37% vs. 11% in the FA and F arms respectively, and larger delta median OS, with 7.3 vs. 6.6 (Δ month) and 7.8 vs. 5.4 (Δ month) in the FA and F arms respectively as well.

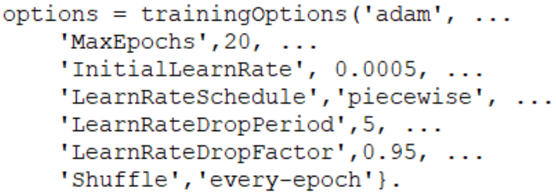
Two comparison trials, the BOND and the 20050181, studied ETS in mCRC patients receiving chemo. + anti-VEGF as a 2nd line treatment. In the BOND trial, optimal ETS criterion "ETS ≥ 10%" was used rather than the "ETS ≥ 5%". In the 20050181 study, the general criterion "ETS ≥ 30%" was used. In terms of delta median OS, the performance of the "ETS ≥ 10%" in the BOND trial and the "ETS ≥ 5%" in the VELOUR trial were similar. But, in terms of response rate, both the BOND and 20050181 trials were much lower, especially the 20050181 trial which used the "ETS ≥ 30%". In studies of first-line chemo. + anti-VEGF treatment (*e.g.*, FIRE-3, PEAK and TRIBE), the ETS criteria "ETS ≥ 20%" and "ETS ≥ 30%" achieved higher response rate, with median and range of 66% (59% - 75%) and 49% (38% - 62%) in the experiment and control arms, respectively.

Overall, three trends of tumor size change patterns could be observed: 1) 1st line treatment introduced faster tumor shrinkage than 2nd line treatment; 2) anti-EGFR treatment introduced faster tumor shrinkage than anti-VEGF treatment; and 3) targeted therapy introduced faster tumor shrinkage than chemotherapy alone. Hence, it is a reasonable trend that, mCRC patients receiving chemo. + anti-VEGF as a 2nd line treatment showed a slow size change pattern in the VELOUR trail, which partially explained the superiority of DL-based criteria over ETS-based criteria.

Supplementary Methods

GoogLeNet Fine-tuning

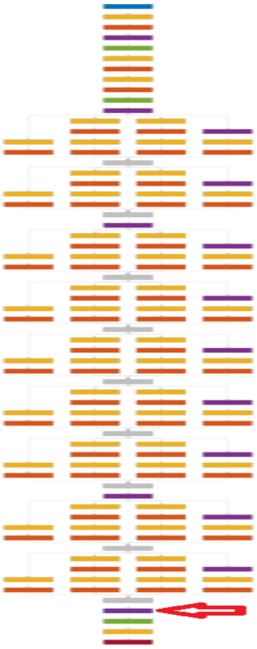
The fine-tuning of GoogLeNet was implemented on Matlab version 9.5. The GoogLeNet we used was downloaded from https://www.mathworks.com/help/deeplearning/ref/googlenet.html. To fine-tune the GoogLeNet to domain of medical images, we created a dataset consisted of lesion images and tissue images. The lesion image set was regarded as positive set and contained totally 1000 lesion images randomly cropped from images of training cohort. Random rotation (range -30~30 degree) and re-scale (rang 0.7 ~1.5) were applied to augment the positive set to 5000 samples. Correspondingly, we constructed a negative set which contained 5000 tissue images randomly cropped from tissue regions. Thus, the downloaded pre-trained GoogLeNet was fine-tuned on the 10000 data set (5000 lesion images vs. 5000 tissue images) to differential lesions from tissues. The parameters for the fine-tuning were as follows,



```
options = trainingOptions('adam', ...
    'MaxEpochs',20, ...
    'InitialLearnRate', 0.0005, ...
    'LearnRateSchedule','piecewise', ...
    'LearnRateDropPeriod',5, ...
    'LearnRateDropFactor',0.95, ...
    'Shuffle','every-epoch'}.
```

Parameters that were not specified were set as default.

The layer for feature extraction was the 'pool5-7x7_s1', as indicated by the red arrow in Supplementary Figure 9.



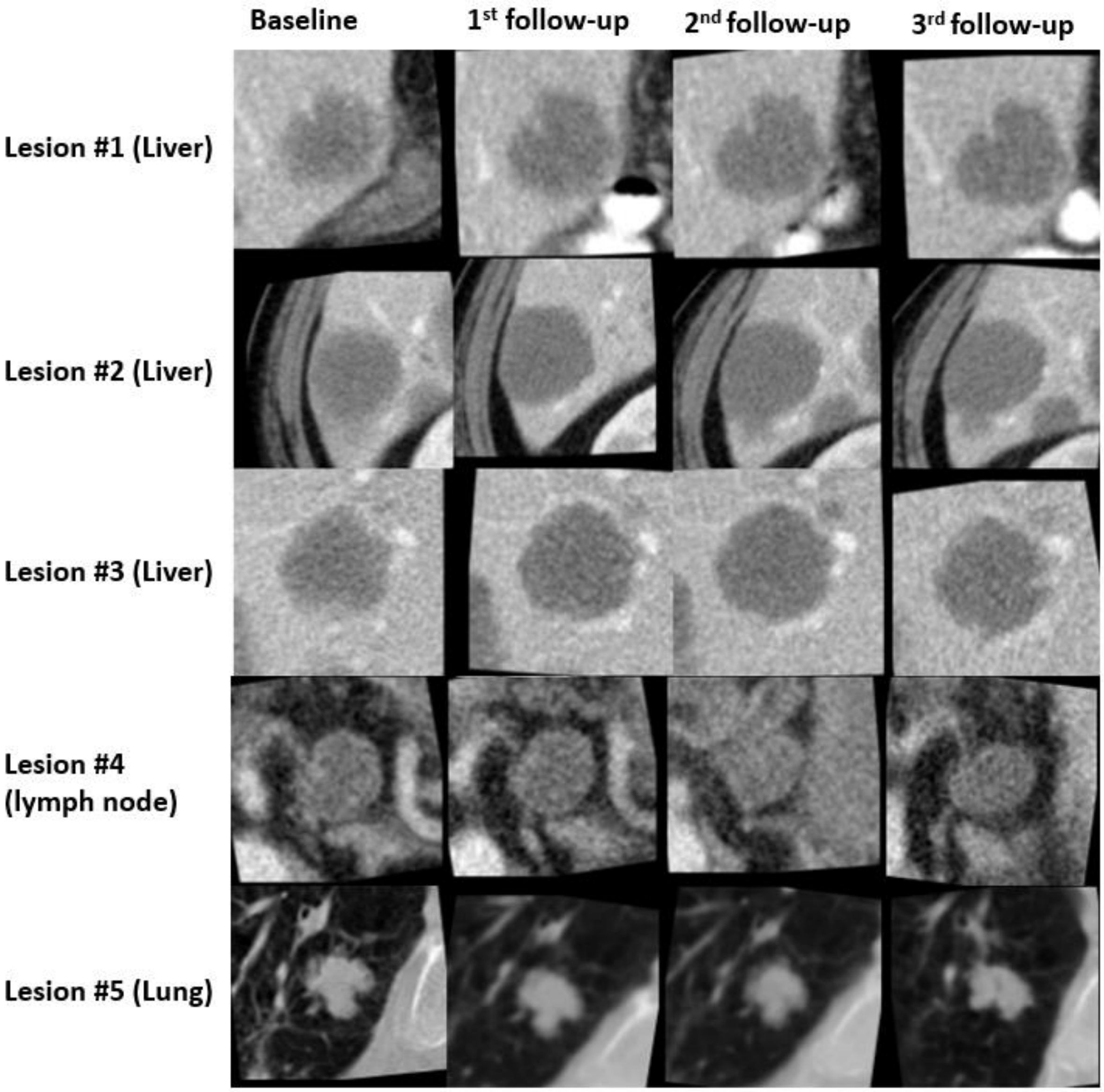
Supplementary Figure 9. The network structure of GoogLeNet. The red arrow indicates where the layer used for feature extraction is. To generate the structure picture in matlab, firstly, install GoogLeNet via the 'Add-on' application; then, load the GoogLeNet into the workspace; and finally, open the GoogLeNet using the 'deepNetworkDesigner' command.

ROI preparation

Four image preprocessing procedures were applied for preparing inputs to DL networks. They were, 1) image spatial normalization, 2) image intensity normalization, 3) determination of region of interest (ROI), and 4) spatial augmentation of ROIs. Some details are as follows.

Firstly, due to the variability in slice thickness and in-plane resolution, all CT images were harmonized to homogenous voxel spacing of 1.0x1.0x1.0 mm^3 by using tri-linear interpolation. Secondly, since lesions in mCRC patients involved multi-organs, cropped ROI images were normalized by using CT window-level. Thirdly, to reduce the computation complexity, image ROIs containing lesions were cropped from the CT images to be as the inputs of network. The ROI of lesion was defined as a box of size 2*d* x 2*d* (where d is the length of diameter measured at baseline) with the center point corresponding to the center of the measurement line at baseline CT scan. It is noted that, lesion ROI at follow-up scan used the same *d* as that at baseline scan. Fourthly, to improve the robustness of the ROIs, in-plane spacing augmentations to ROIs were introduced, including random rotation (-30°~30°), shifting (-0.05*d*~0.05*d*) and scaling (0.95*d*~1.05*d*). The spatial augmentation for training data is ten folds. The selection of augmentation parameters was based on previous study that variability of tumor measurements in mCRC was about 10% [8].

After the image preprocessing, all ROI images were resized to the standard input size for the GoogLeNet, i.e., 224x224 pixel. An example for input of DL network training was presented in Supplementary Figure 10.



Supplementary Figure 10. An example for input of DL network on training. Five target lesions were selected, including three liver lesions, one lymph node lesion and one lung lesion. The CT scans were collected for the patient, including the baseline and the first three follow-ups.

RNN construction and training

The construction and training of RNN were implemented on Matlab version 9.5. The code was available at https://drive.google.com/drive/folders/1LNULttbs9OzjeMzAiY0H705SdveJ175j?usp=sharing

Reproducibility analysis

Two sets of DL prediction scores are compared. The two sets of DL prediction scores are attained via introducing random rotation (-30°~30°), shifting (-0.05*d*~0.05*d*) and scaling (0.95*d*~1.05*d*) to the original ROI. As mentioned in the 'ROI preparation' section above, the selection of augmentation parameters was based on previous study that variability of tumor measurements in mCRC was about 10% [8].

The comparison between the two sets of DL prediction scores is evaluated by the concordance correlation coefficient (CCC) [9].

Supplementary References

1. Piessevaux, H., et al., *Radiological tumor size decrease at week 6 is a potent predictor of outcome in chemorefractory metastatic colorectal cancer treated with cetuximab (BOND trial).* Ann Oncol, 2009. **20**(8): p. 1375-82.
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